

Seroprevalence of *Toxoplasma gondii* in Harbor Seals (*Phoca vitulina*) in Southern Puget Sound, Washington

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ABSTRACT: As part of the Puget Sound Ambient Monitoring Program of the Washington Department of Fish and Wildlife, serum samples from 380 harbor seals (*Phoca vitulina*) were tested for antibodies to *Toxoplasma gondii* in the modified agglutination test (MAT) incorporating formalin-fixed tachyzoites and mercaptoethanol. Antibodies to *T. gondii* were found in 29 of 380 (7.6%) seals with titers of 1:25 in 13, 1:50 in 14, and \geq 1:500 in 2 seals. Results indicate natural exposure of these wild marine mammals to *T. gondii* oocysts.

Sporadic cases of *Toxoplasma gondii* infections have been reported in several species of marine mammals, including beluga whales (*Delphinapterus leucas*; Mikaelian et al., 2000), Atlantic bottlenose dolphin (*Tursiops truncatus*; Cruickshank et al., 1990; Inskoop et al., 1990), striped dolphins (*Stenella coeruleoalba*; Domingo et al., 1992), the spinner dolphin (*Stenella longirostris*; Migaki et al., 1990), northern fur seal (*Callorhinus ursinus*; Holshuh et al., 1985), California sea lion (*Zalophus californianus*; Migaki et al., 1977), and harbor seal (*Phoca vitulina*; Van Pelt and Dieterich, 1973). Diagnosis was confirmed immunohistochemically by reaction to anti-*T. gondii* antibodies in the spinner dolphin (Migaki et al., 1990), Atlantic bottlenose dolphin (Cruickshank et al., 1990; Inskoop et al., 1990), and beluga whale (Mikaelian et al., 2000). *Toxoplasma gondii* antibodies were found in 27% of 22 beluga whales; 3 with titers of 1:25 and 3 with titers of 1:50; the histologically confirmed whale had a titer of 1:25. Recently, Cole et al. (2000) isolated *T. gondii* from tissues of 15 of 67 southern sea otters (*Enhydra leutris nereis*), indicating that a high percentage of these animals have been exposed to *T. gondii* in the wild. In the present study, a serologic survey of antibodies to *T. gondii* is reported in harbor seals in their natural habitat captured in the southern Puget Sound, Washington, from 1994 to 2000.

The Washington Department of Fish and Wildlife (WDFW), as part of the Puget Sound Ambient Monitoring Program (PSAMP), has responsibility for addressing the health and status of marine mammals in Puget Sound, with harbor seals selected as a good indicator species to monitor to meet the goals and objectives of the PSAMP. Harbor seals were selected because they are upper-trophic-level predators that are year-round, breeding residents of Puget Sound, as well as being abundant, widely distributed, and relatively easy to study. The focus of these studies on Puget Sound harbor seals has been directed at seals that use

the Gertrude Island (near McNeil Island) haulout site in southern Puget Sound. This site is the largest harbor seal haulout area in southern Puget Sound, with over 700 seals using it seasonally. As part of ongoing research activities, harbor seals at Gertrude Island have been routinely screened for exposure to various diseases, including brucellosis, leptospirosis, influenza, calicivirus, and morbillivirus.

The following research activities were conducted under Marine Mammal Protection Act (MMPA) Scientific Research Permit nos. 835 and 782-1446. The primary method of capturing harbor seals uses a beach seine technique described in Jeffries et al. (1993). Additional seals were captured by grabbing individuals following either boat or beach rushes. Once captured, seals were placed in individual hoop nets to determine weight, age, sex, and marking. Blood for serologic screening was drawn from the extradural intravertebral vein. For blood collection, serum separator vacutainer tubes were used. Serum was separated as soon as possible into 1–2-ml aliquots and frozen (-20°C) for 2 mo to 6 yr before screening for *T. gondii* antibodies.

Based on known pupping season dates at Gertrude Island and using weight/age correlations for harbor seals from British Columbia (Bigg, 1969), ages of individual harbor seals were classified as nursing pups (<2 mo), weanlings (2–6 mo), yearlings (6–18 mo), subadults (18–48 mo), or adults (>48 mo).

Sera were tested for antibodies to *T. gondii* at 1:25, 1:50, and 1:500 dilutions using the modified agglutination test (MAT) as described by Dubey and Desmonts (1987). Although there is no information on the sensitivity and specificity of MAT for the diagnosis of toxoplasmosis in harbor seals, based on a validation study of MAT in pigs naturally and experimentally infected with *T. gondii* (Dubey, Thulliez, and Powell, 1995; Dubey, Thulliez, Weigel et al., 1995; Dubey, 1997), a MAT titer of 1:25 was considered an indicator of *T. gondii* exposure in harbor seals.

Antibodies to *T. gondii* were found in 29 of 380 (7.6%) seals with titers of 1:25 in 13, 1:50 in 14, and \geq 1:500 in 2 seals (Table I). Antibody titers of 1:50 and higher were found in yearling or older animals, suggesting the antibodies were not acquired from colostrum. These results indicate natural exposure of these marine mammals to *T. gondii* oocysts.

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TABLE I. *Toxoplasma gondii* antibody titers from southern Puget Sound harbor seals, 1994–2000.

Titer	Pup		Yearling		Subadult		Adult		Total
	Fe-male	Male	Fe-male	Male	Fe-male	Male	Fe-male	Male	
<1:25	74	67	26	13	16	20	54	81	351
1:25	1	1	2	0	2	1	2	4	13
1:50	0	0	0	0	3	1	1	9	14
≥1:500	0	0	0	1	0	0	0	1	2
Total	75	68	28	14	21	22	57	95	380

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